

future geometries that would focus at given size cutoffs can be developed. For the particular conditions described herein, $R_c \sim 1$ for a particle diameter of 4.0- μm , a hydraulic diameter of 90- μm and $R_c = 115$. To determine a new geometry for a size cutoff a_c , the experimental parameters can be substituted into the following equation:

$$r_2 \frac{a_c^3}{D_{h2}^4} = 3.2 \times 10^{-4}$$

[0233] Assuming that the radius of curvature is left constant in a new system, the new hydraulic diameter is a function of the desired cutoff:

$$D_{h2} = a_c^{3/4} m^{1/4}$$

[0234] This relation suggests that, for a cutoff of 8- μm , a D_h of 150- μm is required for a channel height of ~ 95 - μm if the width remains constant. This value can be acquired for the scaling of the balance of forces based on a single geometry; determining whether the value converges for separate geometries would provide further support for this approach. Overall, the semi-empirical approach provides the scaling for the ratio of lift to drag forces without providing the magnitude of the individual forces. The speed of focusing, based on the magnitude of lift forces, can be calculated from the fundamental equations.

[0235] Blood cells may be considered to fall on the continuum between rigid particles and deformable droplets; however, since droplets are measured to have a size cutoff similar to rigid particles (3.7 vs. 4.0- μm) the equations presented above are also applicable to cells. The lack of a disparity between deformable droplets and rigid particles suggests similar ratios of inertial lift to Dean drag forces with little additional contributions. The differences, including the relative reduction of smaller particles in fraction 5 of the sorted droplets and the reduced collection range in fraction 4, may be due to forces that are known to act on flexible particles due to deformation in the flow namely, deformation-induced lift forces that additionally act to push deformable particles toward the channel center. These differences, however, should be small in the inertial flows since inertial lift forces have been shown to dominate droplet behavior for small drops or when the viscosity ratio between droplet and suspending fluid is 1 or greater. For highly viscous droplets or cells, the droplet can be expected to behave almost as a rigid particle.

Example 13

[0236] Referring to FIG. 31, inter-particle interactions can play a role in separations, leading to varied behavior for solutions with different total volume fractions. Because the system focuses particles to particular streamlines, one upper limit of particle concentration of $\sim 5\%$ can be calculated, in which all particles are in contact and aligned in a single file. However, even below this concentration, particle-particle effects can limit the degree of focusing. Particles randomly disturb the ideal parabolic flow of the fluid necessary for precise values of inertial lift and Dean drag, as can be seen in FIG. 31. In particular, FIG. 31 illustrated particle concentration effects on ordering. Fluorescent streak images are shown for increasingly concentrated solutions of 9- μm beads flowing through 50- μm focusing channels. Ideal focusing to a

single stream is seen at 0.1% volume fraction of polystyrene beads. Focusing at 1% also remains relatively unperturbed. As concentrations increase further, the focusing is perturbed to a greater extent. A maximum concentration for which focusing could be possible into a single-file stream is $\sim 4\%$ for this geometry, but this calculation assumes particles are touching, and is not physical. Below this concentration focusing is still disturbed because beads change the ideal flow pattern in their vicinity as they traverse the channel.

Example 14

[0237] Referring back to FIG. 23C as well as to FIG. 30, cell viability can be maintained during inertial focusing. Because cells travel at high velocities (~ 0.5 m/s), it is important to evaluate cell viability and damage during this process. It should be noted that cells traveling at steady state with the fluid experience only small normal and shear stresses over their surfaces, while significant forces are briefly felt in the inlet and outlet regions where cells must be accelerated by the fluid. In the systems described herein, the channel width at the inlet can optionally be gradually tapered to minimize this effect. High cell viability is found by vital stain after passing through an exemplary system. Further evidence of little damage is seen in FIG. 30, where scatter plot width and position for blood before processing appears essentially unchanged after passing through the system. Cell debris and blebbing would produce a broader distribution of scatter.

[0238] No significant alterations in cell viability occur after they are passed through the inertial focusing systems described herein at high speeds. Even at average velocities of 0.5 m/s there was no discernable damage to cells (99.0% vs. 99.8% initial viability as measured by using a fluorescent live/dead assay). High cell viability and throughput are critical for applications such as flow cytometry. With inertial self-ordering, clear advantages emerge compared with hydrodynamic focusing used in current flow cytometers. These include (i) a single stream input, (ii) reduction of multiple cells in the interrogation spot because of longitudinal self-ordering, and (iii) angular orientation of nonspherical particles for uniform scatter profiles. Another powerful advantage of this focusing system is that throughput can be easily scaled by parallel channels, as noted above and as shown in FIG. 23C, because additional fluidic channels for the sheath fluid are not required. FIG. 23C demonstrates parallelization of particle alignment for high-throughput analysis. Sixteen parallel channels can be fed from an initially randomly distributed solution of 10- μm particles. A uniformly distributed input can be focused into 16 stable streams at the outlet.

Example 15

[0239] The relative separation performance of the system can also be considered herein. In particular, it is important to characterize the relative performance of the separation embodiments disclosed herein by determining several key figures of merit, which are applicable in different situations. In most cases it is difficult to compare between various techniques, since usually only a single figure of merit that best suits the application is reported. Here four quantifiable measures of performance for separation systems are proposed that would allow easy comparison from device to device: (1) throughput, (2) enrichment ratio, (3) yield, and (4) separation resolution. As trade-offs between the various measures are possible by changing the conditions of separation, these